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RESEARCH ARTICLE

Association of total and dengue-specific IgE levels in the sera with dengue virus inhibition and antibody-dependent enhancement

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ABSTRACT

Dengue, caused by the dengue virus (DENV), poses a significant global health challenge. Effective vaccines and treatments for dengue are lacking due to gaps in understanding its pathogenesis and mechanisms in severe cases. This study investigates the role of immunoglobulin E (IgE) in dengue, focusing on its potential association with virus neutralization and antibody-dependent enhancement (ADE) in DENV replication. Serum samples were obtained from dengue-positive (dengue-IgG positive), SLE (dengue-IgG negative), and control (dengue-IgG and SLE-negative) individuals. SLE sera were included as a control for their high total IgE levels. Total IgE and dengue-specific IgE levels were measured using ELISA. Neutralization assays in Vero and KU812 cells were conducted to assess virus neutralization and ADE, respectively. Dengue-positive and SLE sera showed higher total IgE levels than control sera, although there was no significance seen. Dengue-positive sera showed the presence of dengue-specific IgE, whereas SLE and control sera exhibited negligible levels. Neutralization assay in dengue-positive sera revealed no correlation between IgE levels and virus inhibition. SLE sera, however, demonstrated an inverse correlation between total IgE levels and DENV neutralization, suggesting a potential involvement of total IgE in DENV replication in the context of SLE. Seventy-eight percent of SLE sera, 65% of dengue-positive and 54% of control sera exhibited enhanced virus replication in KU812 cells with serum compared to virus alone, indicating the highest occurrence of ADE in SLE, followed by dengue-positive and control sera. DENV expression in KU812 cells was notably higher in SLE sera, indicating increased ADE risk. However, no association was found between IgE levels and virus expression in KU812 cells across all groups. The inverse correlation between total IgE levels and DENV neutralization in SLE sera suggests that IgE may facilitate virus replication. Further comprehensive exploration is needed to fully understand the role of IgE in dengue pathogenesis.

Keywords: DENV; IgE; ADE; neutralization; mast cells.

INTRODUCTION

Dengue is a hyperendemic arthropod-borne viral disease transmitted by *Aedes* mosquitoes and has become a major public health problem mainly in tropical and subtropical locations (Guzman & Harris, 2015). Dengue virus (DENV) infection is estimated to risk almost half of the world's population per year globally causing 96 million symptomatic cases and approximately 500,000 cases of severe dengue (Bhatt *et al.*, 2013). There are four serotypes of DENV, designated as dengue virus 1 (DENV-1), dengue virus 2 (DENV-2), dengue virus 3 (DENV-3), and dengue virus 4 (DENV-4) (Marshall *et al.*, 2003; Appanna *et al.*, 2012; Inokuchi *et al.*, 2018), each characterized by distinct genetic makeups, with only 60–75% of their amino acid sequences being shared. Malaysia has seen a significant increase in dengue cases since 2000, reaching a notable peak of 130,101 cases reported in 2019, the highest number of dengue cases to date, reflecting that there were 399 infections per 100,000 people. The highest number of deaths recorded was noted in 2015 with 336 deaths (Salim *et al.*, 2021; AbuBakar *et al.*, 2022). The total number of dengue cases climbed to 97.839 in October 2023 compared to the 48,109 cases reported in 2022. In 2020, there were 90,304 instances of DF recorded nationwide in Malaysia; in 2021, there were 26,365 cases; and in 2022, there were about 64,078 cases, according to the Ministry of Health (MoH) Malaysia (https://idengue.mysa.gov.my/ide_v3/pdf/statistik.pdf#page=10, September 2024).

Dengue exhibits a spectrum of clinical manifestations, ranging from asymptomatic cases to mild fever and, in some cases, severe forms leading to death (Kalayanarooj, 2011; Hasan *et al.*, 2016; Khetarpal & Khanna, 2016). Considering that severe disease predominantly occurs in individuals with secondary dengue virus infections, host immunological mechanisms may contribute to the development of dengue haemorrhagic fever (DHF) and/or dengue

shock syndrome (DSS) (Inokuchi *et al.*, 2018). The pathophysiology of severe dengue appears to be multifaceted, involving intricate interactions among viral components, host genetics, and the host's immunologic history, particularly prior infection with the dengue virus (Rothman, 2003). There are several factors that contribute to the risk of contracting severe dengue. Notably, prior exposure to different serotypes of the dengue virus increases the risk, as subneutralizing antibodies may enhance virus infection or trigger pro-inflammatory responses. Pre-existing conditions such as diabetes, hypertension, obesity, cardiac disorders, and allergic asthma are associated with severe dengue, potentially due to heightened baseline inflammation or increased vascular complications. Genetic predisposition, including polymorphisms in HLA types and non-HLA alleles, also plays a role in dengue severity, implicating factors like TNF, IL-10, JAK-1, and TAP (Rathore *et al.*, 2020).

DENV infection has been found to be amplified by subneutralizing levels of dengue-specific antibodies via antibody-dependent enhancement (ADE) (Huang *et al.*, 2016) and there are 2 types of ADE – intrinsic and extrinsic (Halstead, 2014; Narayan & Tripathi, 2020). The Fc receptors (FcR) on circulating monocytes are where the antibody-virus complex attaches. As a result, there is an increased rate of infection and a rise in overall viral replication, hence a risk of developing severe dengue (Whitehead *et al.*, 2007). ADE occurs when, rather than protecting the body against infections, the antibody magnifies and exacerbates the virus infection, making the effects on the host more severe (Marshall *et al.*, 2003; Rothman, 2003; Martina *et al.*, 2009). The inability of the immune response to inhibit DENV replication due to ADE results in elevated viral levels, activating more pro-inflammatory cytokines and chemokines, leading to a 'cytokine storm'. This response significantly contributes to dengue severity, impacting the vascular endothelium and causing heightened permeability, leading to shock and potential organ failure (Chen *et al.*, 2008; Kelley *et al.*, 2012; Modhiran *et al.*, 2015; Srikiatkhachorn *et al.*, 2017).

Previous studies showed that the level of IgE was higher in the post-defervescence serum of dengue patients (Pavri *et al.*, 1979; AbuBakar *et al.*, 1997; Bachal *et al.*, 2015), suggesting its contribution to dengue pathogenesis. In addition, dengue-specific IgE was significantly higher in severe dengue patients (Koraka *et al.*, 2003), proposing the pathogenic role of this antibody (Sanchez *et al.*, 1986), and was also found to be an early predictor of dengue severity (Inokuchi *et al.*, 2018). On the other hand, a previous study showed that sera of SLE patients efficiently neutralized DENV in comparison to healthy individuals, with effects independent of IgG or IgM antibody levels (Zainal *et al.*, 2018). The potential involvement of IgE in the neutralization effects against the virus was suggested as the antibody is known to be elevated in SLE patients' sera in the neutralization effects against the virus. Furthermore, an earlier study showed that human immunodeficiency virus (HIV)-infected children with elevated IgE showed no opportunistic infections or failure to thrive, suggesting the protective role of IgE in HIV disease (Pellegrino *et al.*, 2002). These findings further suggest that IgE could have dual effects in viral infections (Annsley *et al.*, 2024), acting as a double-edged sword.

While the role of IgG and IgM in DENV infection has been studied extensively from protective and pathological aspects (St John, 2013; Lee *et al.*, 2016; Wang *et al.*, 2017; St John & Rathore, 2019), the role of IgE is still indefinite and warrants further research. Here, we aim to investigate the role of IgE in inhibiting virus replication and its effects on antibody-dependent enhancement (ADE) against DENV.

MATERIALS AND METHODS

Study approval

The approval for sample collection and the conduct of experimentations were obtained from the Medical Research & Ethics Committee, Ministry of Health Malaysia (reference number: NMRR-12-1412-1306), Universiti Malaya Medical Centre (UMMC) Medical Ethics Committee (Ethics Committee/IRB Reference Number: 962.6) and Institutional Biosafety and Biosecurity Committee (IBBC) (reference: UMIBBC/NOI/R/FOM/MMB-001/2020). Written informed consent was obtained from each volunteer in this study.

Patient serum samples

The dengue-IgG positive (dengue-positive) and dengue-IgG and SLE-negative (control) sera were obtained from the Tropical Infectious Diseases Research & Education Centre (TIDREC) and the Universiti Malaya Medical Centre (UMMC). SLE patient serum samples were collected from Putrajaya Hospital, and only dengue-IgG negative SLE sera were selected for this study.

Enzyme-linked immunosorbent assay (ELISA)

In order to determine total and dengue-specific IgE levels among the samples, a total of 56 SLE sera, 60 control sera, and 45 dengue-positive sera were used. For the determination of the total IgE level in the serum, the 96-well plate used was coated with anti-human IgE antibody (Sigma Aldrich Chemical, St. Louis, MO, USA) and kept overnight at 4°C. After three washes with 1 x filtered phosphate buffer saline (PBS), the plate was blocked using 5% skimmed milk (Sunlac, Pulau Pinang, Malaysia) for 2 hours at 37°C. Following three washed with 1 x PBS, diluted samples (1 in 10 dilution in 0.5% skimmed milk) were added and the plate was incubated for 1 hour at 37°C. The plate was washed thrice with 1 x PBS, and then anti-human IgE peroxidase-labelled conjugate (Sigma Aldrich Chemical, St. Louis, MO, USA) was added. The plate was incubated for 1 hour at 37°C, followed by triple wash with 1 x PBS. Subsequently, 3,3',5,5'-Tetramethylbenzidine (TMB) substrate (Sigma Aldrich Chemical, St. Louis, MO, USA) was added and incubated for 15 minutes at room temperature. The reaction was stopped using stop solution (Sigma Aldrich Chemical, St. Louis, MO, USA), and the absorbance was measured at 450nm.

To determine the dengue-specific IgE levels using ELISA, the coating and blocking of the plate were performed according to the protocol outlined for the total IgE ELISA. Serum sample at 1:10 dilution was applied to the plate for a duration of 1 hour at 37°C. Following this, unbound antibodies were removed through washing using 1 x PBS thrice, and a mixture of diluted dengue antigens was added to the plate. The plate was then incubated for 1 hour at 37°C. After washing, the plate was further added with anti-dengue horseradish peroxidase (HRP) conjugate (Sigma Aldrich Chemical, St. Louis, MO, USA) to a 1:1 ratio into the diluted dengue antigen and incubated for 1 hour at 37°. The plate was washed three times with 1 x PBS and subjected to the development process as outlined in the total IgE procedure.

The IgE ratio was calculated using the following formula: IgE ratio = (OD sample – OD blank) / (OD blank + [3 x SD]). To ensure consistency and account for any non-specific binding or background noise, each sample's OD value was compared to that of a blank well, which contained all assay components except the serum.

Determination of dengue-specific IgG and IgM levels

SLE sera were screened for the presence of dengue antibodies using the Dengue IgG and IgM capture ELISA kit from Standard Diagnostics, Inc. following the manufacturer's protocol.

Dengue-specific IgG and IgM levels in dengue-positive and control sera were determined using the fluorescent immunoassay system. A total of 10 µL of sera was applied to the Standard F Dengue IgM/IgG fluorescent immunoassay (FIA) kit (SD Biosensor) and subsequently detected using the STANDARD F200 Analyzer machine.

Cell culture

Vero cells from African green monkey kidney epithelial cells utilized in this investigation were obtained from our Swedish partner (Ammerman *et al.*, 2008) that were cultured using Dulbecco's

Modified Eagle Medium (DMEM; Life Technologies, Carlsbad, CA, USA) supplemented with 10% foetal bovine serum (FBS; GIBCO, NY, USA) and kept at 37°C with 5% CO_2 and sub-passaging was done twice a week regularly once the cells become 80-90% confluent.

KU812 cells (Human Peripheral Blood Basophilic Leukaemia Cells) purchased from Icell Bioscience Inc. (iCELL, Shanghai, China, were utilized as mast cells and were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640; GIBCO, NY, USA) medium, which was supplemented with 10% heat-inactivated FBS, 1% non-essential amino acid (NEAA; GIBCO, NY, USA), and 1% L-glutamine (GIBCO, NY, USA). Cells were cultured in a humidified 37°C with 5% CO_2. Weekly sub-passaging of the cells was carried out twice, and the cells were used when approximately 80% confluency was attained.

Viruses and stock preparation

The DENV-1 serotype was used in this study (strain 311012). The virus stocks were prepared by infecting an 80% confluent monolayer of Vero cells with the virus inoculum at 1:40 dilution in serum-free medium. The cells were then subjected to gentle rocking motion at room temperature to allow virus entry. Following absorption, the cell cultures were incubated at 37°C in 5% CO_2 conditions for 7 to 10 days after which the serum-free medium was replaced with DMEM supplemented with 2% FBS. The supernatant was collected and transferred to a 15ml centrifuge tube and centrifuged for 5 minutes at 3500 rpm to remove cells and the cell debris. Subsequently, a 10cc syringe and 0.45µm syringe filter were used to sterilise the supernatant during the filtering process into another 15ml centrifuge tube. After filtration, the supernatant was aliquoted into small tubes and kept in the freezer at -80°C until needed.

The foci-forming assay was used to determine the virus titre. Vero cells were initially seeded overnight in DMEM with 10% FBS supplementation on a 24-well plate at a cell density of $1.5x10^5$ per well, and then incubated at 37°C under 5% CO_2 conditions. The virus was diluted 10-fold in serum-free medium, administered to the cells, and allowed to absorb for 80 minutes at room temperature while being gently rocked. Following the removal of the inoculum from the plates, an overlay medium containing 2% FBS and 1% high-viscosity carboxymethylcellulose (CMC; Sigma Aldrich Chemical, St. Louis, MO, USA) was applied. The cells were then incubated for 96 hours at 37°C with 5% CO_2. Foci staining assay was used to detect viruses.

After the 96-hour incubation, the overlay medium was removed, and the cells were washed thrice using PBS. The fixation of cells was performed using 4% paraformaldehyde (Sigma Aldrich Chemical, St. Louis, MO, USA) diluted with PBS for 20 minutes and subsequently washed thrice with PBS. To permeabilize the cells, 1% Igepal CA-630 detergent (Sigma Aldrich Chemical, St. Louis, MO, USA) was applied for 15 minutes, and the cells were washed thrice again with 1 x PBS. Cells blocking was done using 3% skimmed milk (Sunlac, Pulau Pinang, Malaysia) for 2 hours at room temperature or overnight at 4°C. The cells were then washed thrice with 1 x PBS. Next, the human serum (dengue-positive) was used as the primary antibody and was added at 1:500 dilution to the cells. The mixture was incubated at 37°C for 80 minutes and subsequently washed thrice with 1 x PBS. Following the primary antibody, a secondary antibody (peroxidase-conjugated goat anti-human-IgG) (Sigma Aldrich Chemical, St. Louis, MO, USA) was added and maintained at 37°C for 1 hour. After discarding the previous solution, three 1 x PBS washes were performed on the cells. The immunostained cells were exposed to 3,3'-Diaminobenzidine (DAB) substrate (Thermoscientific, Waltham, MA, USA) for colorimetric detection of the foci, and after 15 minutes at room temperature, the chromogenic development was observed. To calculate the virus titre, the foci were counted and quantified as foci-forming units per millilitre (FFU/ml).

Foci Reduction Neutralization Test (FRNT)

The neutralization capability of sera against DENV was examined using foci reduction neutralization test. In order to analyse neutralization capability among the samples, a total of 56 SLE sera, 21 control sera, and 45 dengue-positive sera were used. Vero cells were seeded overnight at 37°C with 5% CO_2 in a 24-well plate with a cell density of $1.5x10^5$ per well. Patient serum was then heat-inactivated at 56°C before proceeding to 4-fold dilutions (1:80 to 1:5120). The virus stock was diluted to 100 FFU per well and added to the diluted serum. The serum and the virus mixture were incubated at 37°C for 1 hour and then introduced to the seeded cells. Virus absorption was done at room temperature with gentle rocking for 80 minutes. The inoculum was removed from each well and overlay medium was added to each well consisting of DMEM supplemented by 2% FBS and 1% high-viscosity CMC and left to incubate for 4 days at 37°C with 5% CO_2. Foci were stained as mentioned earlier and the virus foci reduction percentage was calculated by counting the virus that had been exposed to the serum of patients and virus that had not been exposed to the serum. The serum dilution that reduced the number of foci to 80% was known as $FRNT_{80}$ and 50% was known as $FRNT_{50}$.

Antibody Dependant Enhancement (ADE) Assay

KU812 cells were used for the assay and a total of 18 samples were used for SLE sera, 11 samples were used for control sera, and 17 samples were used for dengue-positive sera. Cells at $2.5x10^5$ per tube, were suspended in serum-free RPMI medium in 1.5ml centrifuge tubes. Heat-inactivated serum at dilutions of 1:50 and 1:500 in RPMI medium supplemented with 2% FBS was mixed with diluted DENV-1 stock (10 MOI) (final sera dilution of 1:100 and 1:1000, respectively), before being added to the KU812 cells. After a 1-hour incubation at 37°C, the cells were centrifuged, washed gently with serum-free RPMI, added with 2% RPMI, and then incubated for 24 hours at 37°C with 5% CO_2. The cell suspension was subsequently transferred to microcentrifuge tubes, centrifuged, and the supernatant was separately stored from the pellet at -80°C for future use. RNA was then extracted from the cell pellet stored using RNeasy Mini Kit (QIAGEN, Venlo, The Netherlands). The RNA was used for qRT-PCR using primers as listed in the manufacturer protocol for SensiFAST SYBR Hi-Rox One Step Kit (Meridian Bioscience, Ohio, USA). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene was used for this qRT-PCR as the control.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.0 software (Applied Biosystems, CA, USA). Differences in the IgE ELISA ratio between groups were analyzed using Dunnett's multiple comparisons test for total IgE and dengue-specific IgE ratios. The association between DENV neutralization percentage and IgE ELISA ratio was determined using Spearman's correlation analysis. The results of the qRT-PCR were obtained through StepOne software version 2.3 and analyzed in GraphPad Prism 9.0 to examine the presence of ADE and its association with the neutralization capabilities of the sera using Dunnett's multiple comparisons test. All analyses were considered as significant when $P< 0.05$.

RESULTS

Total IgE and dengue-specific IgE antibodies detection using enzyme-linked immunosorbent assay (ELISA)

We examined the level of both total IgE and dengue-specific IgE of the serum samples using ELISA. SLE sera showed higher total IgE levels in comparison to the control sera (Figure 1), even though no significance was noted. This aligned with previous studies suggesting elevated total IgE levels in SLE patients, which is often indicative of greater disease severity (Dema *et al.*, 2014; Lamri & Charles, 2020). Dengue-positive samples also showed higher total IgE levels compared to the control sera, nearly matching the level observed in SLE sera (Figure 1) but showed no significant difference.

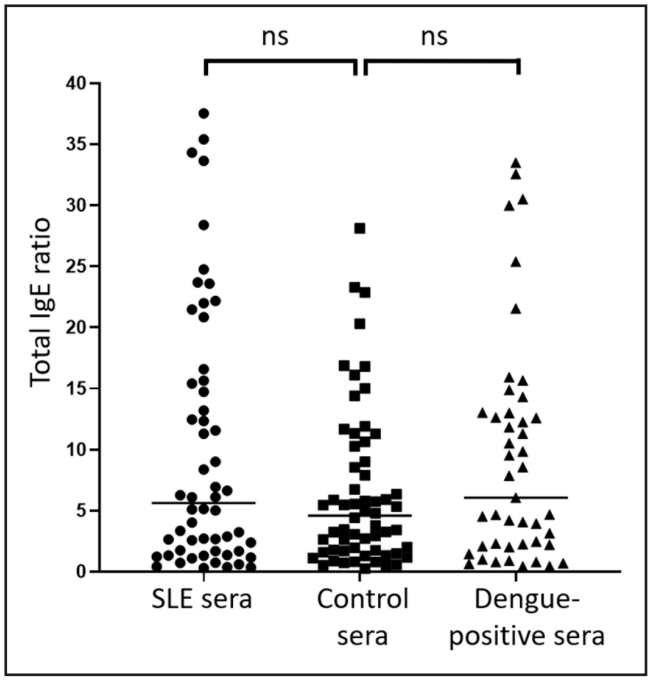

Figure 1. Total IgE antibody level in SLE sera, dengue-positive and control sera. Total IgE antibody levels in SLE, dengue-positive, and control sera were measured using ELISA. Correlations were assessed by Dunnett's multiple comparisons test. Significant differences between the groups are indicated as ns (not significant).

In the detection of dengue-specific IgE, we observed a generally low to negligible levels with less than 1.0 dengue-specific IgE ratio in both SLE and control sera, consistent with their seronegative status of dengue (Figure 2). However, one SLE sera showed a high level that could be due to cross-reactions with other flavivirus. Dengue-specific IgE levels, with a mean ratio of 1.58, were detected only in dengue-positive sera, as expected due to the previous exposure of these individuals to dengue.

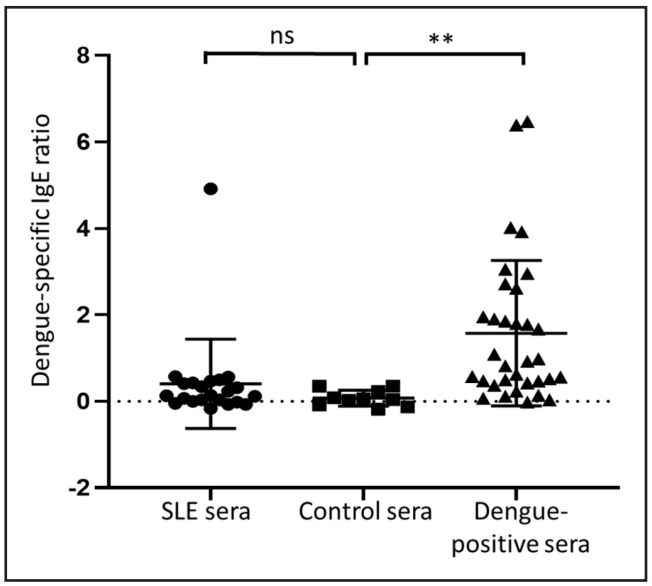

Figure 2. Dengue-specific IgE antibody level in SLE sera, control sera and dengue-positive. Dengue-specific IgE antibody levels in SLE, dengue-positive, and control sera were measured using ELISA. Differences between the groups were assessed using Dunnett's multiple comparisons test and are shown as ns (not significant) and **P<0.005.

Association between IgE (total or dengue-specific) antibody levels and the neutralizing capability of sera against DENV-1 using FRNT

To investigate the potential involvement of IgE in neutralizing the dengue virus, we examined the association between IgE (total or dengue-specific) antibody levels and the neutralizing capability of all sera against DENV-1.

We observed a 50% neutralization capability against DENV across all dilutions. The FRNT assay showed 75% of seronegative SLE sera, 100% of dengue-positive sera, and 95% of control sera in the lowest dilution of 1/80 (Supplementary Figure S1A). At a dilution of 1/320 (Supplementary Figure S1B), the FRNT assay revealed that 46% of seronegative SLE sera, 100% of dengue-positive sera, and 81% of control sera exhibited 50% neutralization capacity against DENV. Subsequently, at a dilution of 1/1280 (Supplementary Figure S1C, the FRNT assay demonstrated that 14% of seronegative SLE sera, 91% of dengue-positive sera, and 81% of control sera had 50% neutralization capacity against DENV. Lastly, at the maximum dilution of 1/5120 (Supplementary Figure S1D), the FRNT assay showed that 21% of seronegative SLE sera, 82% of dengue-positive samples, and 76% of control sera displayed 50% neutralization capacity against DENV-1. Association of total IgE antibody ratios against the inhibitory percentage of DENV-1 were analysed next. In SLE sera at dilutions 1/320 (Figure 3B), we observed significant inverse correlation between total IgE levels and the inhibitory percentage of DENV (P=0.0213), where higher total IgE levels are associated with decreased inhibition of the virus. While a similar inverse correlation pattern can be observed across all dilutions, no significant correlation was noted at serum dilutions of 1/80 (P=0.1018), 1/1280 (P=0.1236), and 1/5120 (P=0.4830) (Figures 3A, 3C, 3D). For dengue-positive sera (Figure 4), no correlation between the total IgE antibody level and the DENV-1 inhibition percentage was consistently observed across all dilutions of the sera (1/80; P=0.7318, 1/320; P=0.8442, 1/1280; P=0.8844, 1/5120; P=0.6717). For control sera (Figure 5D), there was significant association observed in the highest dilution of 1/5120 (P=0.0495). No correlation between total IgE levels and DENV-1 inhibition percentage was evident across all the other dilutions of control sera (1/80; P=0.5338, 1/320; P=0.2714, 1/1280; P=0.4955) (Figures 5A, 5B, 5C).

We further explored the association between dengue-specific IgE antibody ratio and the DENV-1 inhibition percentage in dengue-positive sera, the only type of sample that showed presence of the antibody. Results showed no significant correlation across all dilutions of the sera (1/80; P=9325, 1/320; P=0.6079, 1/1280; P=0.5188, 1/5120; P=0.6258) (Figure 6).

Antibody-dependent enhancement (ADE) of DENV in KU812 cells and its association with total IgE antibody level

KU812 cells were inoculated with a mixture of DENV-1 (MOI of 10) and serum diluted to 1/100 or 1/1000. Control of this assay was the inoculation of the DENV-1 at an MOI of 10 without serum. The results demonstrated that 15 out of 18 (83%) SLE (Figure 7A), 12 out of 17 (71%) dengue-positive (Figure 7B), and 6 out of 11 (55%) control sera (Figure 7C) showed higher virus expression in KU812 cells with serum compared to the virus without serum (virus control). Among sera that exhibited ADE, 9 out of 15 (60%) of SLE (Figure 7A), 7 out of 12 (58%) of dengue-positive (Figure 7B), and 6 out of 6 (100%) of control sera (Figure 7C) showed higher virus expression at serum dilution of 1:100 compared to 1:1000. However, statistical analysis revealed that these differences were not statistically significant. When compared among the groups, SLE sera showed higher virus expression in both 1/100 (Figure 8A) and 1/1000 (Figure 8B) serum dilution compared to dengue-positive and control sera.

Correlation analysis between DENV-1 expression in KU812 and total IgE antibody levels showed no association at both 1/100 and 1/1000 dilutions in SLE (1/100; P=0.7416, 1/1000; P=0.8293) (Figure S2), dengue-positive sera (1/100; P=0.1793, 1/1000; P=0.1348) (Figure S3), and control (1/100; P=0.1107, 1/1000; P=0.8818) (Figure S4).

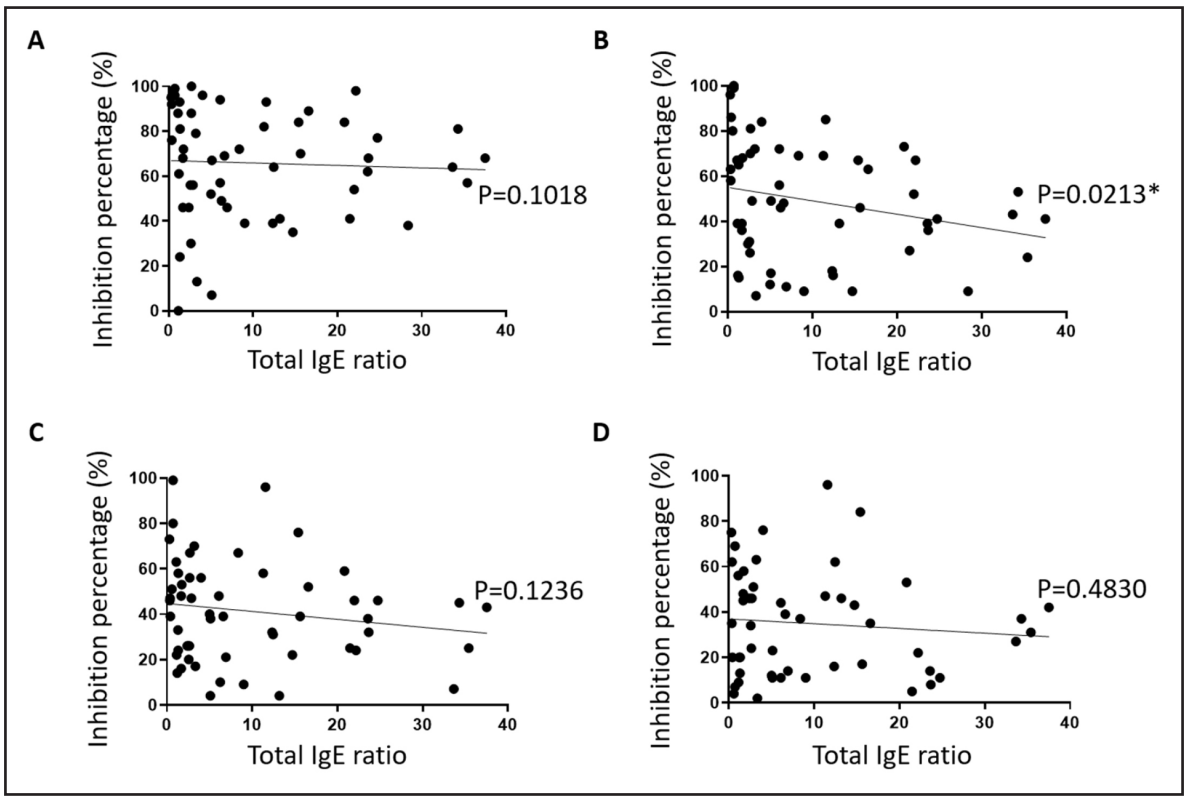

Figure 3. Association of total IgE antibody level in SLE sera with neutralization level against DENV-1. Heat-inactivated SLE (dengue-IgG negative) (n=56) sera were diluted into (A) 1/80, (B) 1/320, (C) 1/1280, (D) 1/5120 and incubated with DENV-1 for 80 minutes. The incubated solution was then added to Vero cells and virus titers were determined using focus-forming assay. Spearman's correlation test was used to assess the relationship between total IgE antibody levels in SLE sera and the neutralization level against DENV-1. Significant association is indicated with *P<0.05.

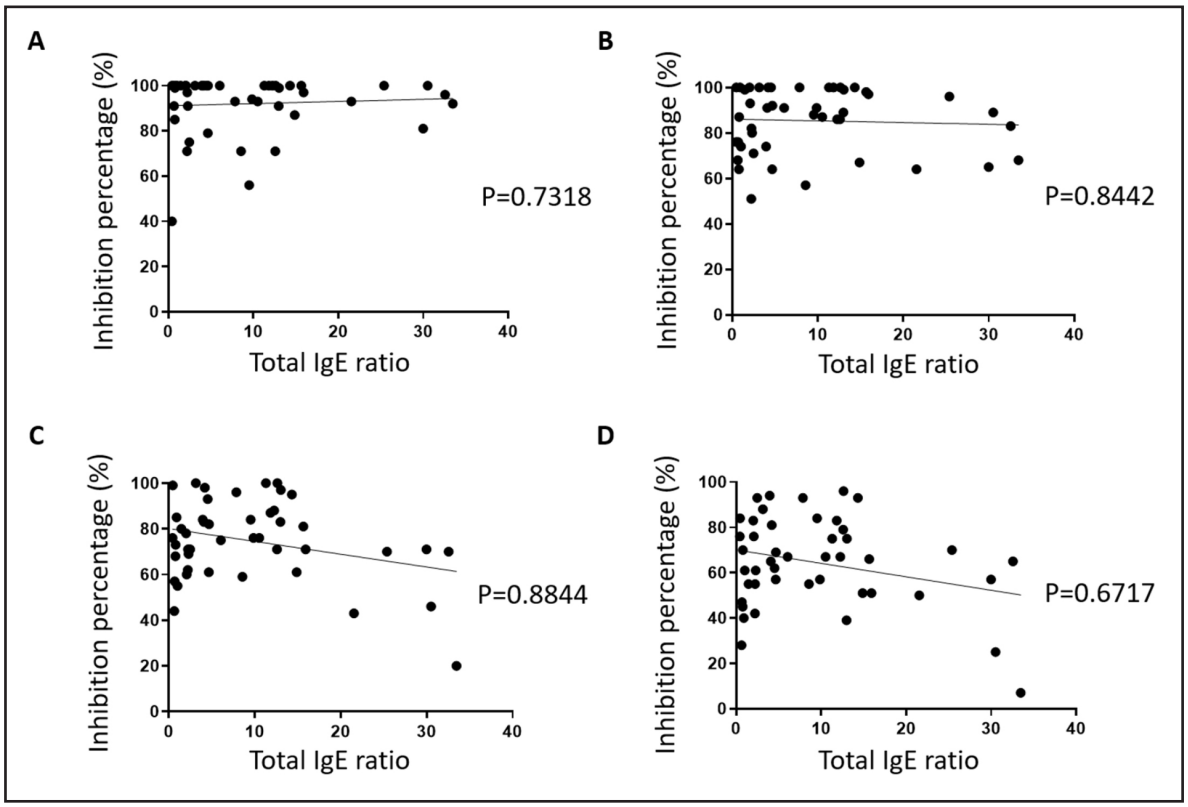

Figure 4. Association of total IgE antibody level in dengue-positive sera with neutralization level against DENV-1. Heat-inactivated dengue-IgG positive (no known SLE and dengue IgG and/or IgM positive) (n=45) sera were diluted into (A) 1/80, (B) 1/320, (C) 1/1280, (D) 1/5120 and incubated with DENV-1 for 80 minutes. The incubated solution was then added to Vero cells and the virus titers were determined using focus-forming assay. Spearman's correlation test was used to assess the relationship between total IgE antibody levels in SLE sera and the neutralization level against DENV-1.

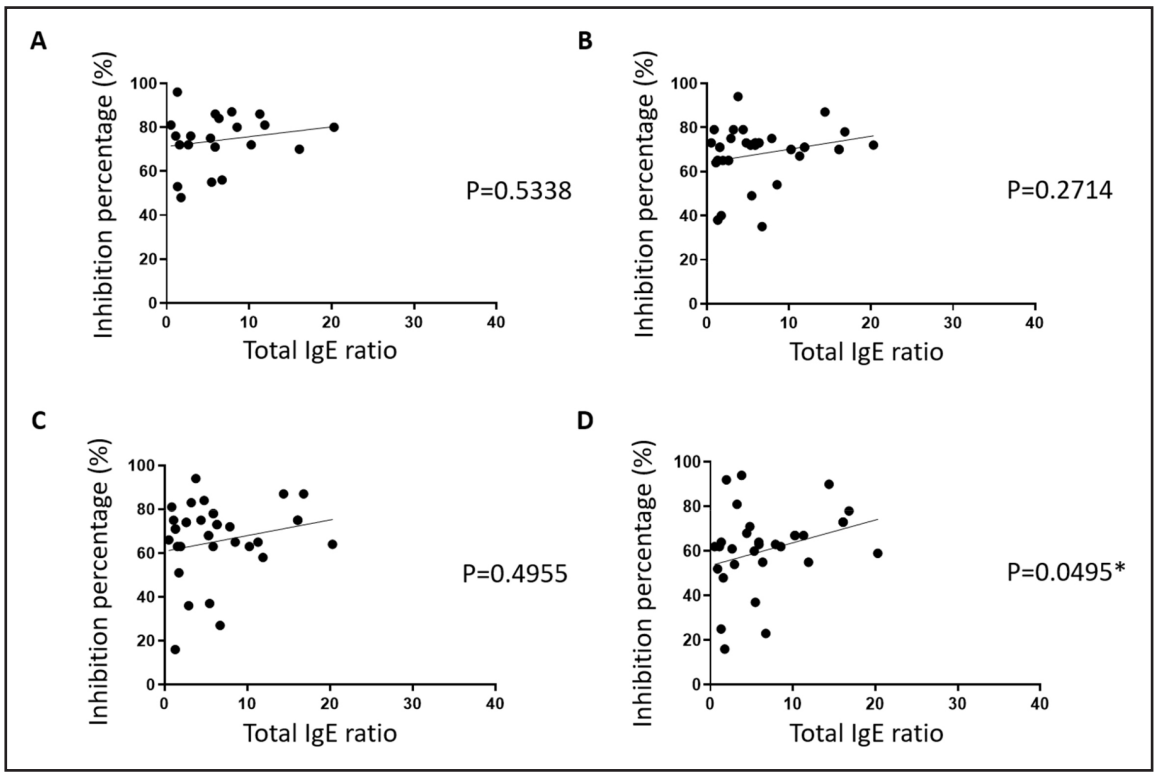

Figure 5. Association of total IgE antibody level in control sera with neutralization level against DENV-1. Heat-inactivated dengue-IgG and SLE-negative (n=21) sera were diluted into (A) 1/80, (B) 1/320, (C) 1/1280, (D) 1/5120 and incubated with DENV-1 for 80 minutes. The incubated solution was then added to Vero cells and the virus titers were determined using focus-forming assay. Spearman's correlation test was used to assess the relationship between total IgE antibody levels in SLE sera and the neutralization level against DENV-1. Significant association is indicated with *P<0.05.

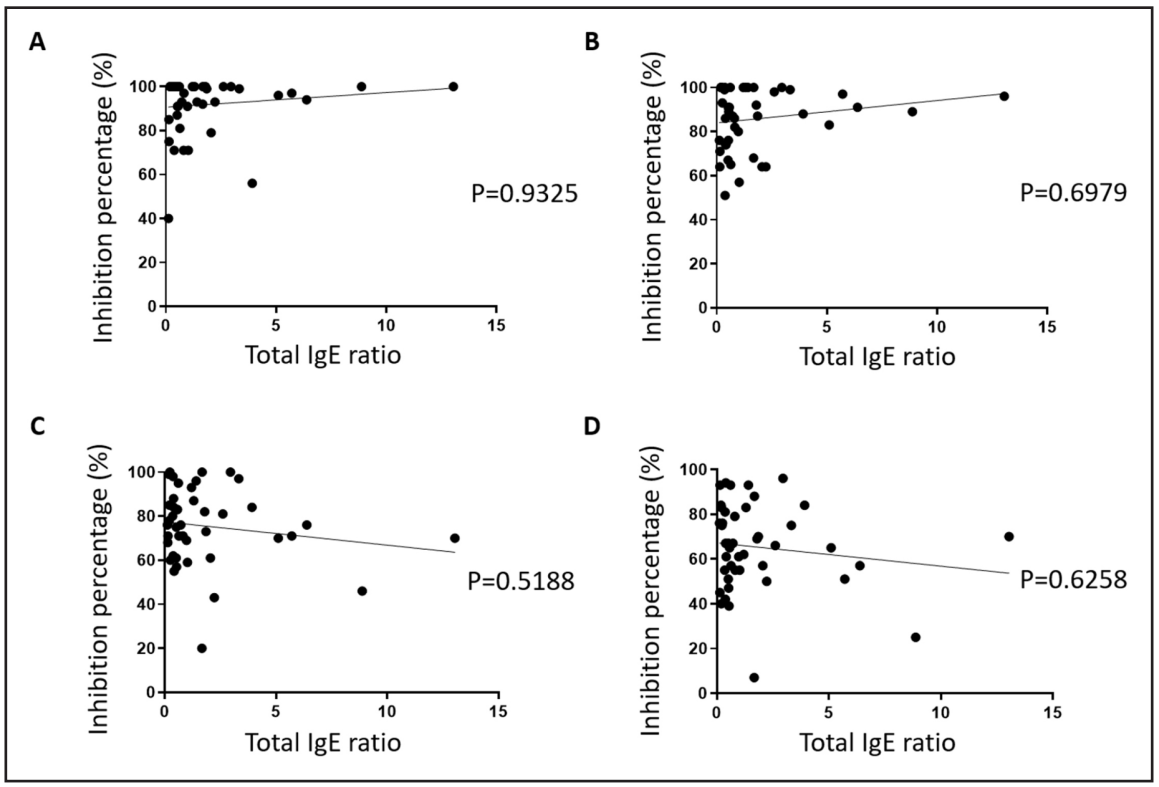

Figure 6. Association of dengue-specific IgE antibody level in dengue-positive sera with neutralization level against DENV-1. Heat-inactivated dengue-IgG positive (no known SLE and dengue IgG and/or IgM positive) (n=45) sera were diluted into (A) 1/80, (B) 1/320, (C) 1/1280, (D) 1/5120 and incubated with DENV-1 for 80 minutes. The incubated solution was then added to Vero cells and the virus titers were determined using focus-forming assay. Spearman's correlation test was used to assess the relationship between total IgE antibody levels in SLE sera and the neutralization level against DENV-1.

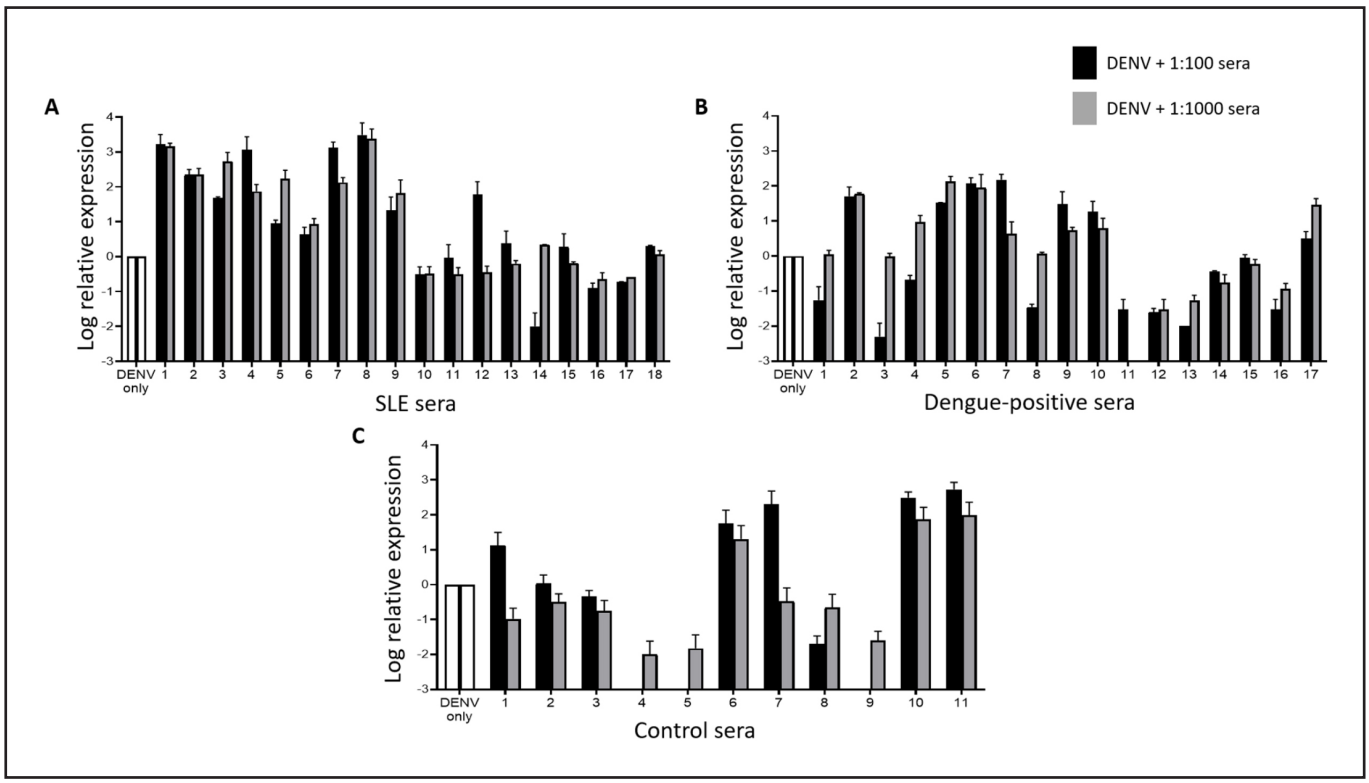

Figure 7. Log relative expression of DENV-1 in SLE sera, dengue-positive and control sera. SLE (A), dengue-positive (B), and control sera (C) were diluted to 1:100 and 1:1000 and subjected to ADE assay against DENV using KU812 cells. RNA was extracted and qRT-PCR was performed to measure the relative expression of DENV-1. GAPDH was used as the reference housekeeping gene for normalization. The data are presented as log-relative expression levels of DENV-1.

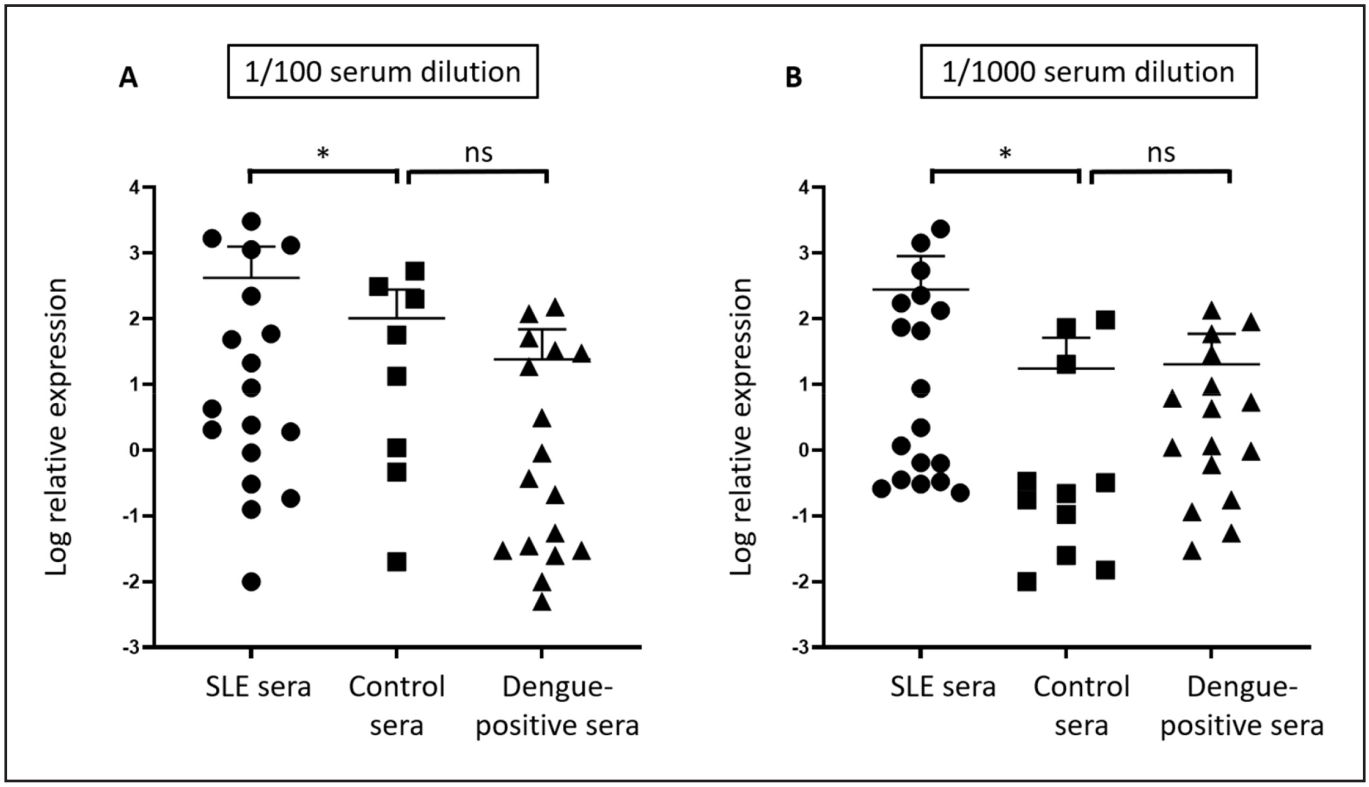

Figure 8. Comparison of log relative expression of DENV-1 in SLE sera, dengue-positive and control sera. SLE, dengue-positive, and control sera were diluted to 1:100 (A) and 1:1000 (B) and subjected to ADE assay against DENV using KU812 cells. RNA was extracted and qRT-PCR was performed to measure the relative expression of DENV-1. The log-relative expression levels of DENV-1 were compared between the groups. Correlations were assessed by Dunnett's multiple comparisons test. Significant differences between the groups are indicated as ns: not significant and *P<0.05.

DISCUSSION

Previous study showed elevated total IgE in SLE patients with a self-reported history of childhood-onset allergies, including asthma, hay fever, or eczema (Biagini *et al.*, 2008). Another study demonstrated a strong association between IgE autoantibodies and increased disease activity and nephritis in SLE patients (Dema *et al.*, 2014). Additionally, IgE autoantibodies in SLE patients correlated with disease severity, attributed to the aberrant interferon response activation through pDC and TLR-9-mediated sensing of DNA in the phagosome (Henault *et al.*, 2016). These findings correlate with our result of slightly elevated levels of total IgE in SLE (Figure 1). The unexpected moderate elevation of total IgE in dengue-positive sera is somewhat notable, especially considering the limited number of studies reporting a similar outcome. A study among San Andres Island's population was the first to describe elevated total IgE levels in the sera of individuals exposed to dengue compared to dengue seronegative individuals (Míguez-Burbano *et al.*, 1999), supporting our finding. Another study showed a significantly high level of both total and dengue-specific IgE in patients with dengue virus infection in comparison to non-dengue patients. Dengue-positive sera showed increased levels of dengue-specific IgE as predicted, but SLE and control sera showed little to no dengue-specific IgE (Figure 2). The relatively high dengue-specific IgE levels were mainly found in dengue haemorrhagic fever and/or dengue shock syndrome patients (Koraka *et al.*, 2003). These findings suggest an association between IgE levels and disease progression in dengue-positive individuals, a correlation previously observed in studies with SLE sera. These results also imply the potential impact of IgE levels in dengue or severe dengue detection.

Our finding showed possible involvement of total IgE in assisting DENV replication in individuals with SLE (Figure 3), supporting the potential pathological role of IgE in dengue. While no other studies directly relate IgE with the pro-viral replication of DENV, a study demonstrated that autophagy facilitates DENV replication in mast cells, a cell that has high affinity to IgE antibodies (Fang *et al.*, 2014). IgE was also associated with severe progression of dengue (Míguez-Burbano *et al.*, 1999; Koraka *et al.*, 2003). Significant association was observed between total IgE level and dengue with pruritus, indicating the relationship between allergy and dengue severity (Kien *et al.*, 2020). Mast cells-derived mediators has also been linked with the development of DHF and DSS (Furuta *et al.*, 2012). Association between total IgE and dengue-specific IgE with DENV inhibition, however, were not observed in dengue-positive samples, suggesting that the significant inverse association may be exclusive in SLE sera. The absence of correlation between total IgE and dengue-specific IgE antibody levels in dengue-positive and control sera, along with the inverse correlation of total IgE levels in SLE sera with virus inhibition, suggests that IgE levels do not contribute to the neutralization of DENV. Instead, they might assist in DENV replication in individuals with SLE. The control sera showed considerable viral inhibition even though they did not have SLE and were ELISA-classified as dengue-negative. This rather conflicting finding might be explained by exposure to other flaviviruses in the past (Oliveira *et al.*, 2019; Yong *et al.*, 2022) that could cross-react with DENV.

Previous study demonstrated ADE during DENV infection in KU812 cells with an elevated level of vasoactive cytokines (Fang *et al.*, 2014). Antibody-enhanced binding of all four dengue virus serotypes to KU812 cells was also observed with the increased production of inflammatory mediators including IL-1, IL-6, and CCL5 (Brown *et al.*, 2006). We investigated the occurrence of ADE of DENV infection in KU812 cells. The results showed very low to almost no virus replication detected in the KU812 cells infected with DENV-1 without sera at an MOI of 10, while virus can be detected in the KU812 cells infected with DENV-1 mixed with some of the sera (Figure 7). This correlates with the previous study that showed no DENV infection without sera in KU812 cells and positive DENV replication with sera, suggesting ADE, where antibodies in the sera facilitate with the infection of DENV-1 in KU812 cells (Brown *et al.*, 2006). Similarly, some of the sera samples in our study also showed results comparable to a previous study (Brown *et al.*, 2006), where higher DENV replication was expressed in lower sera dilution (higher concentration of antibodies) compared to higher sera dilution (lower concentration of antibodies). A previous study by King et. al also showed that ADE was expressed in both dilutions, however, the expression was greater in the more diluted sera, which is similar to some of our sera samples (King *et al.*, 2000). The expression of DENV-1 was significantly higher in SLE sera than in control and dengue-positive sera at both dilutions, suggesting a higher risk of ADE in SLE patients (Figure 8). A case report documented a patient with active lupus nephritis who developed dengue haemorrhagic fever (DHF), leading to subsequent bleeding and fluid overload (Vidanapathirana & Atukorala, 2023). The generation of autoantibodies and immune complexes in SLE has been found to alter the immunological environment, affecting the capacity of antibodies to neutralize and thereby raising the risk of ADE (Justiz Vaillant *et al.*, 2024). We, however, found no correlation between ADE and total IgE levels in SLE and dengue-positive sera. It is possible that specific factors or components within the SLE sera contribute to this phenomenon, enhancing viral replication through mechanisms that are not solely related to total IgE levels. Previous study showed a heavier role of IgG in ADE activation compared to IgE in dengue, due to the dominant effect of FcγRII in comparison to FcεRI, where the blockade of FcγRII with monoclonal antibody significantly hindered DENV binding to KU812 cells (Brown *et al*., 2006). We, however, do not find any association between all sera IgG level and ADE in this study (data not shown).

Our study used only one serotype, DENV-1, rather than all four serotypes. This limitation led to results that were not as comprehensive. Additional limitations of our study included time constraints and limited resources. Challenges encountered during the study included obtaining a high titre of virus MOI. Some of our control sera showed both neutralizing capability and ADE, which contributed to many insignificant findings when compared to SLE and dengue-positive sera. This might be due to possible exposure of these sera to other flaviviruses that could induce cross-reaction against DENV. Furthermore, our results may not be generalizable to certain age groups and locations, as our investigation was conducted with sera without considering the target age group or the specific environment with high dengue virus transmission.

In conclusion, we observed moderate elevation of total IgE in dengue-positive and SLE sera, suggesting that total IgE may be a useful biomarker for detecting dengue or severe dengue. The reversed association of total IgE and DENV inhibition in SLE patients implies possible involvement of total IgE in assisting DENV replication. Furthermore, the significantly higher expression of the virus in KU812 cells in SLE sera compared to other groups suggests an increased risk of ADE in DENV infection among SLE patients. The observed correlations between antibody levels, virus neutralization and ADE indicate a complex relationship that warrants further exploration.

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Disclosure

There are no conflicts of interest in relation to this manuscript.

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